

Smart EPU Software

A platform for automated screening and data acquisition in cryo-EM single particle analysis

Thermo Scientific™ Smart EPU Software offers enhanced automation capabilities for high-quality data acquisition in single particle analysis (SPA) workflows. It provides real-time, AI-assisted decision-making algorithms to aid beginners and to offer a higher degree of flexibility for advanced users.

Although recent advancements in instrumentation have made it more efficient to collect large amounts of data, the quality of single particle analysis data still largely depends on the expertise of the microscope operator. Smart EPU Software is an innovative platform that combines well-known features of previous EPU Software releases with novel, easy-to-use tools designed to further automate the software:

- Simple microscope preparation for optimized imaging
- Intuitive data collection setup
- Optimized Autoloader usage for improved screening and data collection
- Automated selection of imaging areas and self-monitoring of imaging conditions for higher efficiency

By encoding expert knowledge into neural networks, Smart EPU Software can automatically recognize and categorize areas in cryo-electron microscopy (cryo-EM) grids that are suitable for imaging, discarding those that would lead to suboptimal results. These AI-driven algorithms assist in the selection of imaging areas to evaluate sample quality, saving valuable time and helping to refine your imaging choices.

Smart EPU Software can include two optional components for real-time image processing: EPU Quality Monitor (EQM), which performs motion correction and contrast transfer function (CTF) determination to dynamically adjust data collection, and Embedded CryoSPARC Live™ Software, a powerful image processing package that offers 2D and 3D information on the molecular content in the sample, online feedback loops, and the optimization of ongoing acquisitions. When combined with Smart Plugins, you can increase data quality and efficiency for an even faster setup.

Additionally, an open application programming interface (API) provides maximal flexibility for expert users by allowing AI algorithms to influence and automate a number of EPU acquisition strategies.

Key Benefits

EPU 3 Software with image filtering simplifies operation and supports training across all compatible instruments.

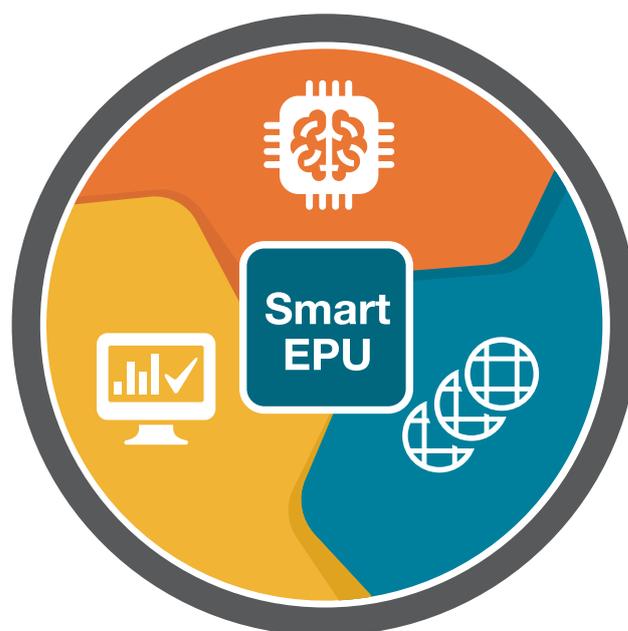
Embedded CryoSPARC Live Software or EPU Quality Monitor analyzes images on the fly and integrates with Thermo Scientific™ CryoFlow™ Software to enable remote access for rapid quality evaluation.

EPU Multigrid Software allows you to queue multiple grids and automate feedback loops when integrated with Smart Plugins for faster setup, efficient microscope use, and improved data quality.

AI-powered Smart Plugins provide real-time feedback, automating data collection without manual monitoring.

Customize, share, and develop your own plugins via an open application programming interface, enabling targeted sample analysis.

Ongoing updates and professional maintenance are supported by our expert development, service, and application specialist teams.



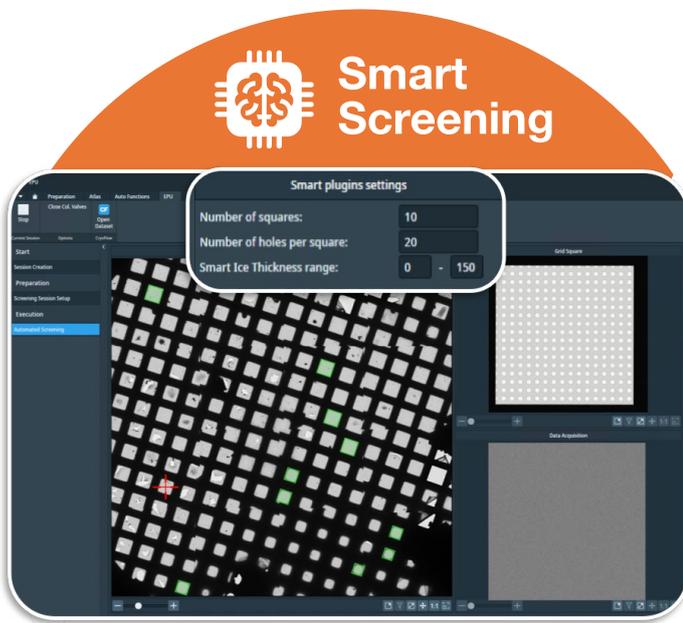


Figure 1. Smart EPU Software can support fully autonomous smart screening with AI-based plugins that automate and enables unattended screening sessions of multiple grids.

EPU 3 and EPU Multigrid Software

EPU Software is a popular choice for the cryo-EM SPA workflow largely due to its robust performance and intuitive interface. Unexpected interruptions of automatic acquisitions are reduced, allowing thousands of images to be collected in just a few hours for structural determination of proteins at up to atomic resolution. EPU Software has continuously evolved for more than a decade, and its most recent version — EPU 3 Software — is designed to further facilitate its use, saving you time and effort.

As a core component of Smart EPU Software, EPU 3 Software includes functions for all the steps of automated SPA data collection, including:

- Saving and loading all relevant preferences that define an acquisition on the Home Page
- Preparing optical sets
- Recording low-magnification grid atlases
- Testing and calibrating autofunctions (Optimize Optics, focus, astigmatism, eucentricity, and Volta phase plate activation)
- Setting up and running acquisition sessions

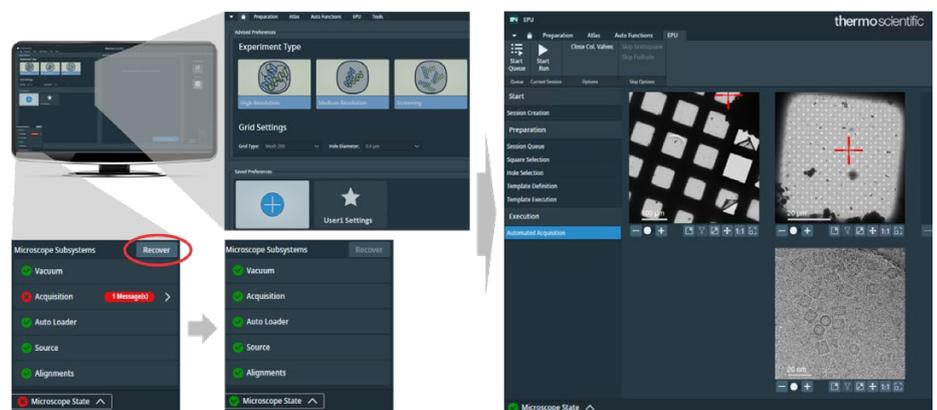


Figure 2. The Smart EPU Software user interface presents the main steps of an acquisition run in a logically organized sequence. Recently implemented features include a Traffic Lights panel to monitor the microscope state and a Home Page to start sessions faster with pre-defined parameters, as well as a routine for optics optimization.

These tasks are divided into subtasks, such as hole selection, for which Smart EPU Software contains semi-automated functions, or relevant AI elements for full automation of critical steps.

Smart EPU Software is also equipped with advanced features such as aberration-free image shift (AFIS), which is designed to enhance data acquisition efficiency and accuracy. AFIS allows for large beam shifts without introducing off-axis coma and astigmatism. By shifting the image beam instead of the stage, AFIS acquires data in clusters and improves throughput by enabling faster acquisition of high-quality images. The Fast Acquisition Mode centers groups of foil holes and uses AFIS to inspect all foil holes in these centered group, significantly reducing the time required for data collection. Extended AFIS mode, available on the Thermo Scientific™ Krios™ 5 Cryo-TEM, further increases the size of foil hole clusters up to 20 μm from the center of the group, resulting in up to 25% greater throughput.

Preferential orientation is a known issue in single particle analysis, resulting in anisotropic density maps. To address this, EPU 3 Software includes a tilted acquisition scheme using AFIS to accelerate the collection of specimens tilted up to 40 degrees. By combining several datasets recorded at different angles, it is possible to generate meaningful particle models. EPU 3 Software also supports plasmon imaging, which is particularly beneficial for examining variations in ice thickness on holey gold grids.

Another component of Smart EPU Software is EPU Multigrid Software, which maximizes efficiency in Autoloader-equipped microscopes. It facilitates the logistics of sample screening and data acquisition for multiple grids, increasing the number of datasets you can generate in each working day, including unattended overnight and weekend runs. Overall, EPU Multigrid Software reduces idle beam time, thereby increasing microscope productivity.

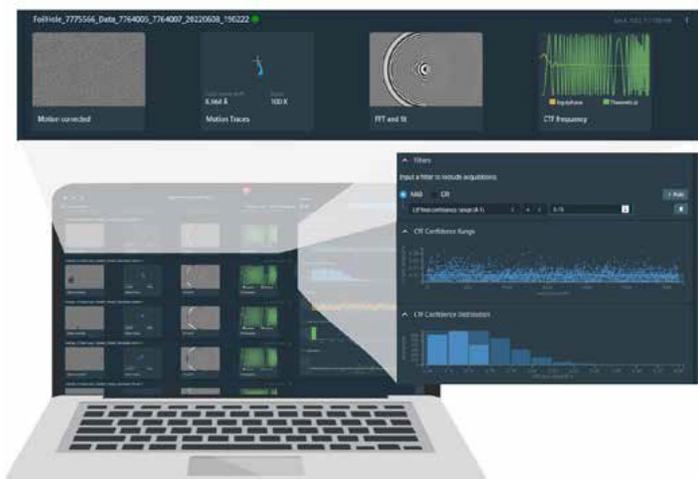


Figure 3. EPU Quality Monitor and CryoFlow Software allow EPU data to be accessed remotely for evaluation and sharing.

EPU Quality Monitor and Embedded CryoSPARC Live

Smart EPU Software can be configured with two alternative, integrated programs for on-the-fly monitoring of data quality: EPU Quality Monitor (EQM) Software or Embedded CryoSPARC Live Software.

EQM Software provides real-time information about image quality throughout the entire data collection process. Drift and optics-related information derived from motion correction and CTF-estimation are accessible for each image immediately after acquisition.

Embedded CryoSPARC Live Software offers 2D and 3D information derived from the data while collection is still ongoing, providing real-time monitoring of both image and biological sample quality. It also enables the adjustment of imaging and processing parameters at any time, increasing overall data quality while allowing you to discriminate between successful and unsuccessful experiments objectively and rapidly. An initial high-resolution

reconstruction of the sample can be achieved while still collecting data. If a poor-quality specimen is identified, Embedded CryoSPARC Live Software ensures you do not waste further beam time and storage, while also pointing towards potential areas to improve during sample preparation, such as preferred orientations or low ligand-binding stoichiometries.

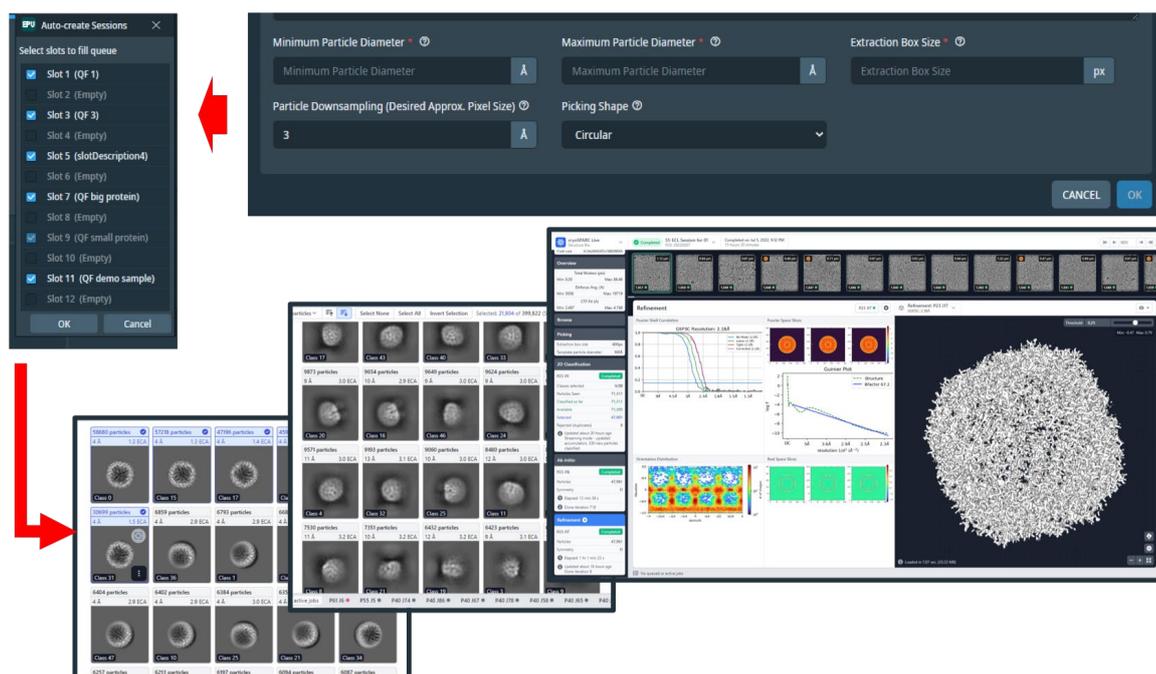


Figure 4. On-the-fly SPA with Embedded CryoSPARC Live Software enables automated image processing using five user-provided parameters in EPU 3 Software. These parameters are applied to each selected grid in EPU Multigrad Software, allowing unattended data processing, up to 2D classification, for multiple grids. With further user interventions, the analysis can generate 3D structures.

In Smart EPU Software, the information gathered from both programs can be used to adjust acquisition parameters not only manually (as has been the case previously), but also automatically with Smart Plugins. With either EQM or Embedded CryoSPARC Live Software, acquisition parameters can also be adjusted at any time. Notably, the filtering capabilities of EQM Software can exclude poor-quality images from image processing, reducing cost and time.

Smart Plugins

Smart Plugins are a set of decision-making algorithms for improving microscope efficiency. Smart EPU Software presents a set of innovative AI/machine-learning plugins that automate steps that previously needed manual actions during the setup of EPU sessions. Specific Smart Plugins are provided for the classification of grid squares according to ice quality, foil hole identification, foil hole selection based on ice thickness, and curation of initial selections to avoid highly contaminated foil holes. Neural networks have been trained to automate these laborious and repetitive tasks, helping you to determine favorable conditions for further collection of large datasets.

The image evaluation from EQM or Embedded CryoSPARC Live Software can also be accessed with Smart Plugins in order to modify the course of a run. This makes it possible to automate feedback loops based on quantitative parameters derived from images. The feedback plugins included with Smart EPU Software consist of Smart Focus for autofocus stabilization, Smart Stage Time to adjust stage waiting time based on drift motion, and Smart Grid Skip for skipping suboptimal grid squares based on the information limit.

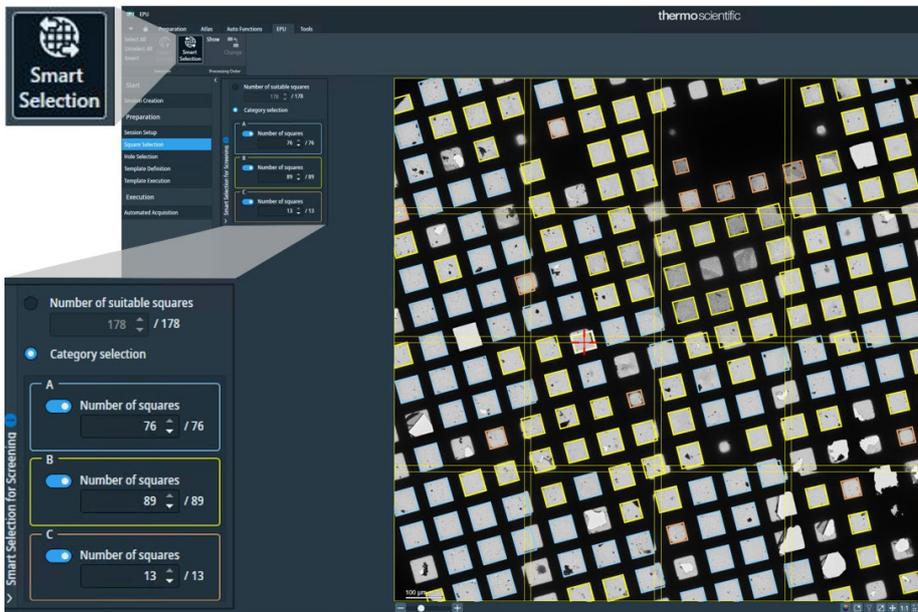


Figure 5. Smart EPU Software can automatically acquire atlases for specimens in multiple slot positions and automatically classify grid squares into groups based on their apparent size and other ice quality metrics.

Open API

An important feature of Smart EPU Software is its open application programming interface (open API), which allows advanced users to develop their own plugins to evaluate microscope outputs and influence Smart EPU Software according to specific needs. Open API accelerates innovation and the exchange of setups within the broader community.

CryoFlow Software

The robust integration of Smart EPU Software components is facilitated by Thermo Scientific CryoFlow Software, a platform for data exchange management, data analysis, and visual reporting. CryoFlow Software ensures smooth and secure transfer of images and associated metadata between components, including recorded images accessible to EQM and Embedded CryoSPARC Live Software, Smart Plugins, and the automated transfer of decisions to the microscope in real-time. CryoFlow Software presents all results in a comprehensive and graphical interface. The Discovery Viewer add-on traces the recording position for each high-resolution image, including the corresponding grid square and foil hole. This makes it possible to compare results taken at different ice qualities within CryoFlow Software.

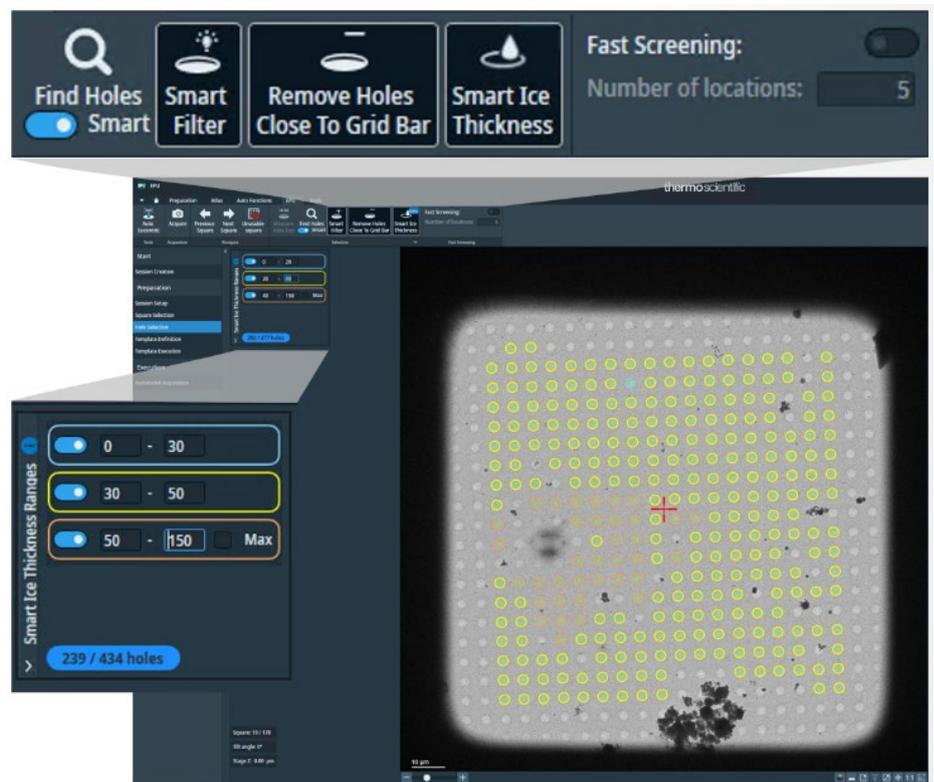


Figure 6. In Smart EPU Software, three Smart Plugins can be applied consecutively to the same grid square image to automatically identify foil holes, curate the initial hole selection to discard empty or ice-contaminated holes, and classify the remaining holes by their predicted thickness. The resulting hole groups are indicated by discreet colors based on adjustable thickness thresholds.

A dedicated interactive panel filters datasets based on these parameters and lets you easily organize, view, and share SPA projects.

The web interface of the CryoFlow Software imaging server can be accessed through both computers and mobile devices connected to a local network. Privacy and permission settings define who can access individual experiments.

Smart EPU Software is ready to use immediately after installation and is supported by the Thermo Fisher Scientific instrument service team,

digital solutions engineers, and application specialists. We are committed to continuously improving the Smart EPU Software experience.

Smart EPU Software with EPU Quality Monitor is pre-installed on Thermo Scientific™ Tundra™ Cryo-TEMs and is available for Thermo Scientific™ Glacios™ and Krios™ Cryo-TEMs (Table 1).

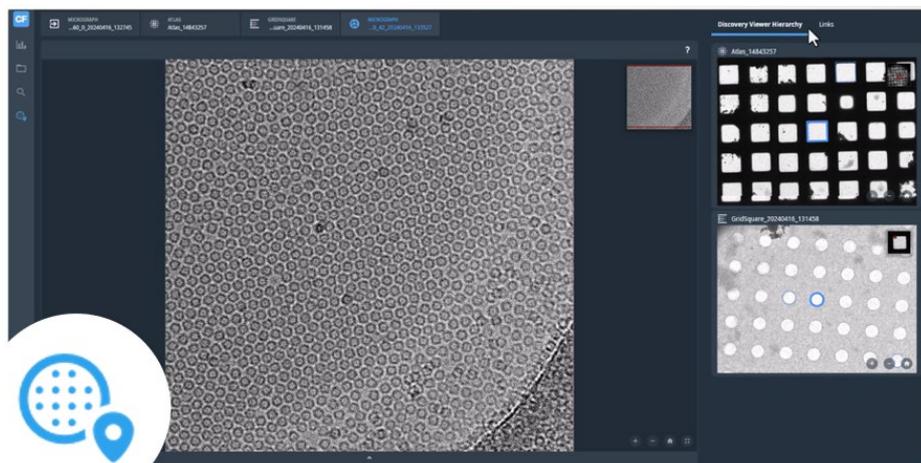


Figure 7. CryoFlow Software offers tools, such as the Discovery Viewer, to help you easily visualize data and metadata associated with your SPA data collection. The hierarchical organization of the data allows you to trace the position of each high-resolution image on the grid. You can easily use this information to guide selection of sites for further imaging.

Smart EPU Software is also available as an upgrade for any existing platforms and integrates the latest technological advances, such as the Thermo Scientific™ Falcon™ 4/4i Direct Electron Detector (including Electron Event Representation) and Thermo Scientific™ Selectris™ Imaging Filters (as well as the Gatan BioContinuum/K3 Filter) to significantly accelerate automated data acquisition.

Summary of Smart EPU Software solutions

Component	Capability	Description	Tundra Cryo-TEM	Glacios Cryo-TEM	Krios Cryo-TEM
EPU 3 Software	Data acquisition	Data acquisition automation	Included	Included	Included
EPU Multigrid Software	Scheduling of data acquisition	Set up unattended and/or multi-day data collection runs	N/A	Optional	Included
EPU Quality Monitor Software	Real-time data evaluation	Real-time motion and CTF correction	Included	Optional	Optional
Embedded CryoSPARC Live Software	Real-time data evaluation	Real-time motion and CTF correction, particle picking, and 2D/3D particle averaging	Optional	Optional	Optional
Smart Plugins	Decision algorithms*	Automatic selection of imaging areas and adjustment of microscope parameters using real-time data from EQM or Embedded CryoSPARC Live Software	Included	Optional	Optional
Open API	Decision algorithms	Tailored scripting to adjust microscope parameters	Included	Included	Included
CryoFlow Software	Data management	Data integration for workflow optimization, reporting, and data sharing	Included	Included	Included

Table 1. Overview of Smart EPU Software components and availability with Thermo Scientific Cryo-TEMs. All Smart EPU Software components require Windows 10. No additional hardware needed for recent instruments. Consult with your sales account manager for retrofit requirements.

*All Available Smart Plugins (Smart Selection of Grid Squares, Smart Hole Finder, Smart Ice Thickness Prediction, Smart Filter, Smart Focus, Smart Stage Time, and Smart Skip Grids Squares) depend on the availability of a real-time data evaluation component.

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